January 17, 1991

Subject: **Final Technical Report** for ONR Grant N00014-90-J-1471 (UW Budget #62-5021) Multidisciplinary Studies of Archaebacteria from Submarine Hydrothermal Environments

From: John A. Baross, School of Oceanography WB-10

During the period of this contract we have completed three submersible expeditions to the Endeavour region of the Juan de Fuca Ridge and a surface ship operation to study the microbiology and chemistry in thermophilic environments and oceanic plumes associated with this vent field. Research was concentrated on isolation and characterization of extremely thermophilic bacteria, their activity under *in situ* conditions, and the rates of microbial oxidation of methane in both the vent field and in the plumes. We have obtained evidence that thermophilic bacteria are abundant at the Endeavour vent field. Viable hyperthermophiles (growth above 80°C) were isolated from sulfides associated with smokers, within flange structures, on surfaces and in guts of animals living on sulfide structures, and in hot waters. In addition, biochemical evidence including total DNA, lipid and protein content was used to show presence of intact bacteria even in extreme environments where attempts at culturing hyperthermophiles, such as areas in the flange where the temperature was greater than 200°C, were unsuccessful.

More than 40 strains of hyperthermophilic archaebacteria were isolated. These include anaerobic heterotrophs, methanogens, and a slow growing group of organisms capable of utilizing low molecular weight organic acids. Characterization of 4 of these organisms have been completed and several others have been partially characterized. The temperature growth range for most of the strains is between 70 and 110°C, all require salt, and most strains require elemental sulfur. The heterotrophs have complex nutritional requirements including all amino acids and some vitamins. A defined medium has been delineated for one of these heterotrophs making it possible to design substrate transport and genetic experiments. This strain, designated ES-4 and related to the Pyrococcus/Thermococcus group, is the designated organism in my laboratory for further studies related to pressure and temperature effects on growth and substrate transport, and effects of heat shock on protein patterns and increased survival at high temperatures. ES-4 is barotolerant and grows optimally at 250 atm pressure and grows much more rapidly

at pressures between 200 and 400 atm at its upper temperature range for growth. Pressure/temperature effects on the growth of three strains of vent thermophiles have clearly demonstrated that each strain responded uniquely. ES-4 has also been shown to produce unique proteins at the upper and lower temperature growth ranges. Similarly, the molecular structure of the membrane lipids also change at different growth temperatures (work in collaboration with D. White and D. Hedrick, University of Tennessee). We have also used lipid analyses for both identification of archaebacteria and their relative biomass in hydrothermal vent samples.

Methane oxidation studies at vent environments during the 1987 and 1988 expeditions to the Endeavour have been completed and manuscrips published or submitted for publication. These studies showed that the maximum methane oxidation in vent waters occurred in stable environments where methane levels are still significantly elevated over ambient seawater levels. No oxidation occurred in the discharging vent water or in the lower portion of the buoyant plume despite high methane levels. The highest methane oxidation rates measured in the entire hydrothermal vent system were associated with the outer surfaces of vent invertebrates such as limpet shells and tubeworm tube. Moreover, we have demonstrated that higher methane oxidation rates occur under in situ hydrostatic pressure than at one atmosphere.

Four graduate students were supported by this contract to complete the microbiology portion of this program. Marie de Angelis completed her PhD in 1990 on methane oxidation and Ralph Pledger will complete his PhD in May, 1992 on characterization of vent hyperthermophiles and pressure/temperature effects on their growth and uptake of substrates. Ivan Tosques will be completiing his MS degree in February, 1992 on iron reducing anaerobic thermophiles and Jim Holden should complete his MS by June, 1992 on heat shock proteins in ES-4.

Manuscrips published, in press, or submitted resulting from this ONR Contract

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Pledger, R. J. and J. A. Baross. Preliminary description and nutritional characterization of an chemoorganotrophic archaebacterium growing at temperatures of up to 110°C isolated from a submarine hydrothermal vent environment. J. Gen. Microbiol. 137:203-211, 1991.

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Baross, J. A. The origin and early evolution of life in the subcrustalenvironments of submarine hydrothermal vents: An assessment. In S. A. Macko, M. Engel and E. L. Shock (eds.), Survivability of Organic Matter at High Temperature: Implications for Life. Geochimica et Cosmochimica Acta. Invited manuscript in preparation, 1992.

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Delaney, J. R., R. E. McDuff, J. A. Baross, M. D. Lilley and M. S. Goldfarb. A newly recognized submarine hydrothermal environment: Flange-trapped pools of buoyant 350°C fluid. Earth Planet Sc. Lett., in preparation, 1992.

Pledger, R. J., B. Crump and J. A. Baross. Temperature and hydrostatic pressure effects on growth of three strains of hyperthermophilic archaebacteria isolated from submarine hydrothermal vent environments, in preparation, 1992.

Straube, W. L., J. W. Deming, C. C. Somerville, R. R. Colwell and J. A. Baross. Particulate DNA in smoker sluids: Evidence for existence of microbial populations in hot hydrothermal systems. Appl. Environ. Microbiol. 56:1440-1447, 1990.

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N00014-90-J-1471
October 8, 1991

Mr. Donald W. Allen, Director
Grant and Contract Services
3935 University Way, NE
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Seattle, Washington 98105

Dear Mr. Allen:

Grant N00014-90-J-1471, (UW Budget #62-5021) expired September 30, 1991. Research was performed under the direction Dr. John A. Baross, School of Oceanography. In order to complete the University's obligation under this agreement, please assure that the following documents are submitted:

___ Final Voucher along with the Contractor's Release of the Government and the Contractor's Assignment of Refunds, Rebates and Credits.

___ Final report required by the Patent Clause. (DD Form 882 enclosed). Negative reports are required for contracts.

PI X Final Technical Report, Performance Report, or transmittal document for the Final Technical Report. Documentation furnished must show date report was provided both to the Scientific Officer, and to Defense Technical Information Center (DTIC).

EICC X Final Property Inventory, with requested disposition. Provide a separate listing for Government-furnished and contractor-acquired property.

GCA X Final 'Financial Status Report' (S Form 269), and any break-down of grant costs.

Any funds excess to the requirements of the agreement should be promptly reported to the contract administrator.

If you have any questions concerning closeout requirements, please do not hesitate to call us at (206) 543-2656.

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JOHN A. BAROSS
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Sincerely,

ROBERT J. SILVERMAN
Administrative Grants Officer